3. If a pseudo-homozygote type for a locus was generated, evidence and exemplar samples amplified with the same primer sequence can be used for comparison.
   a. Identifiler has the same primer sequences as Cofiler and Profiler Plus; however, these sequences differ in Minifiler.
   b. Therefore, the results from amplification with Identifiler may not be reproducible when compared with those of Minifiler.

4. If the same locus is amplified using a multiplex system with primer sequences that differ, it is possible to obtain a heterozygote type in one multiplex and the pseudo-homozygote in the second. The heterozygote type is the correct type and should be reported.

VII. Guidelines for Interpretation of Results

The purpose of these guidelines is to provide a framework which can be applied to the interpretation of STR results in casework. The guidelines are based on validation studies, literature references, some standard rules and experience. However, not every situation can be covered by a pre-set rule. Equipped with these guidelines, analysts should rely on professional judgment and expertise.

A. First evaluate the profile in its entirety to determine whether the sample is composed of one or more contributors.

1. **For Low Template (LT-DNA) samples, refer to the interpretation section of the manual for samples amplified with 31 cycles.**

2. **A High Template DNA (HT-DNA) sample profile can be considered to have originated from a single source if:**
   a. Excluding stutter and other explainable artifacts, the sample does not demonstrate more than two labeled peaks at each locus.
   b. The **peak height ratio (PHR)** at each heterozygous locus is above 60.5% for samples amplified with the AmpFlSTR Identifiler® kit for 28 cycles. Note the PHR of a heterozygous pair is determined by dividing the height of the shorter peak (in RFUs) by the height of the taller peak (in RFUs) and expressing the result as a percentage.

Back to Table of contents

c. If the PHR falls below 60.5% at a locus, consider whether this may be due to a primer binding site mutation, degradation, the amount of template DNA, or extreme allele size differences. Under these circumstances a sample may be considered single source and heterozygote pairs may be assigned even if greater imbalance is observed.

d. If the sample profile complies with the conditions above but three labeled peaks are present at a single locus, the DNA contributor may be tri-allelic at that locus.

3. If an additional allele is present at only one or two loci, these alleles may be the result of a low level mixture detected only at those loci. The source of these allele(s) cannot be determined. The sample may be interpreted according to the guidelines for single source samples.

a. No conclusions can be drawn regarding the source of these alleles that cannot be attributed to Male or Female Donor X.

b. Moreover, no comparisons can be made to this allele(s).

4. **Samples that do not meet the single source criteria listed above should be considered mixed samples.**

B. DNA results may be described in one of three categories, designated as "A", "B", or "C".

1. **Samples and/or components of samples with data at all targeted loci should be categorized as "A". This category includes the following:**

a. Single source samples with labeled peaks at all loci and no peaks seen below the detection threshold.

b. The major and the minor contributors of mixtures where DNA profiles are determined at all targeted loci including those loci assigned a "Z" if the "Z" designation was due to potential allelic sharing.

c. The major contributors of mixtures where the DNA profile of the major contributors were determined including those loci assigned a "Z" if the "Z" designation was due to potential allelic sharing, but the DNA profile of the minor contributors were not determined.

Back to Table of contents

d. Mixtures where the DNA profiles of the contributors were not or could not be determined and no peaks were seen below the detection threshold.

2. All samples or components of samples that are not categorized as "A" described above or "C" described below may be considered "B". This encompasses a wide continuum of samples including the following:

a. Single source samples with labeled peaks at fewer than all targeted loci and/or peaks below the detection threshold.

b. The major and/or the minor contributors to mixtures where DNA profiles were determined at less than the targeted number of loci. At least 4 complete loci or at least 5 loci including those assigned a "Z" if the "Z" designation was due to potential allelic sharing or dropout, should have been determined.

c. Mixtures where the DNA profiles of the major and the minor contributors could not be determined and peaks were noted below threshold, or allelic dropout is suspected.

3. **Samples and/or components of samples categorized as "C" should not be interpreted or used for comparison. This category includes the following:**

a. Too few peaks labeled

i. Single source HT-DNA samples with fewer than eight labeled peaks over four STR loci

ii. HT-DNA single source profiles with fewer than eight alleles over four loci

iii. Single source LT-DNA samples with fewer than eight labeled peaks over six STR loci in the composite

iv. LT-DNA single source profiles with fewer than eight assigned alleles over six loci

v. Single source YSTR data samples with fewer than four alleles over four YSTR loci

vi. Mixed HT-DNA samples with fewer than 12 labeled peaks over six STR loci

vii. Mixed LT-DNA samples with fewer than 12 labeled peaks over eight STR loci in the composite

Back to Table of contents

viii. Mixed samples where after deconvolution of the major contributor, there remain fewer than eight labeled peaks that cannot be attributed to the major component. In this situation, the remaining alleles should not be used for comparison.

*Note: If after deconvolution, the deduced profile of the major contributor has fewer than eight assigned alleles over four STR loci for HT-DNA samples or eight assigned alleles over six STR loci for LT-DNA samples, the sample should be interpreted as a mixture for comparison only.

b. Too many peaks labeled
   i. Mixed HT-DNA samples that show seven or more labeled peaks (repeating or non-repeating) at two or more STR loci
   ii. Mixed LT-DNA samples that show seven or more labeled peaks at two or more STR loci in the composite

c. Other sample characteristics
   i. Mixed HT-DNA samples that show excessive number of peaks below the detection threshold seen over many loci
   ii. Mixed LT-DNA samples that show excessive number of non-repeating peaks above or below the detection threshold seen over many loci
   iii. Mixed HT-DNA samples with template amounts less than 150 pg and mixed LT-DNA samples with template amounts less than 20 pg that show drastic inconsistencies between replicates.

d. Use the Not Suitable for Comparison/Inconclusive documentation to record the reason for categorizing a sample as category “C”. For mixtures which can be deconvoluted for the major contributor, but are not suitable for comparison to the minor contributor, as described above in 3a iv, document the reason.

NOTE: The interpretation protocols detailed below and in the ID31 interpretation section accommodate samples from categories A and B.

Back to Table of contents

C. Interpretation of single source samples.

1. For LT-DNA samples refer to the interpretation section of the manual for samples amplified with 31 cycles.

2. HT-DNA samples may be used if they fulfill the concordant analysis and duplicate rule. Refer to the "General Guidelines for DNA Casework".

3. If multiple injections are generated for a given PCR product, and/or if multiple amplifications were performed, for each locus select the injection and/or amplification that shows the greatest number of labeled peaks.

4. For replicate results check for consistency and assign the allele(s). If results are not consistent between the replicates, a locus may be inconclusive or assigned a "Z".

5. Peak height imbalance is a feature of heterozygotes. Refer to tables 10a and 10b for OCME Identifiler® validation results. For single source samples, heterozygote pairs may be assigned even if greater than average imbalance is observed. Consider the potential contribution of stutter if one labeled peak is in the stutter position of the other.

6. When a single labeled peak is present, consider the potential for a false homozygote. It is possible that allelic dropout occurred.

   a. Apply caution when interpreting samples with labeled peaks below 250 RFU or samples that show a pattern of degradation. Regardless of the height of labeled peaks at other loci, if the peak in question is less than 250 RFU, this could be a false homozygote and a "Z" should be assigned to the locus to indicate the possibility of a heterozygote.
   b. Consider whether the single labeled peak is at a large and/or less efficient locus. In Identifiler, these loci are: CSF1PO, D2S1338, D18S51, FGA, TH01 and D16S539. Consider also whether the single labeled peak is in the last labeled locus of each color. For example, in Identifiler, if CSF has no labeled peaks and a single labeled peak is seen at D7S820, this could be a false homozygote.

Back to Table of contents

D. Mixture Deconvolution

1. For LT-DNA samples refer to the interpretation section of the manual for samples amplified with 31 cycles.

2. There are several categories of mixtures that may be deconvoluted.

a. The major contributor is unambiguous.

b. The major contributor and the minor contributor can be deconvoluted using the specific guidelines described in the following sections.

c. The major contributor can be deconvoluted using the specific guidelines described in the following sections, but the minor contributor cannot.

d. The major contributor or the minor contributor can be deconvoluted using an assumed contributor and the specific guidelines described in the following sections.

3. Take the following general guidelines into consideration when evaluating a mixed sample.

a. For a deduced profile, a locus may be deemed inconclusive for the deduction; however, this data might still be useful for comparison.

b. Caution should be used when deconvoluting the following types of samples:

i. Mixtures with DNA template amounts between 100 pg and 250 pg.

ii. Three person mixtures. These mixtures should only be deconvoluted if one or more contributors are very minor.

iii. If multiple amplifications are performed, and at a locus, one allele is seen in just a single amplification.

c. The major contributor may be determined using the specific guidelines in the following sections without using an assumed contributor.

i. Mixture ratios and potential allele sharing can be used to evaluate genotype combinations; however, the PHRs of the allelic pairs should meet the specific guidelines described in the following sections.

Back to Table of contents

ii. For potential allele sharing, consider all possible genotype combinations at each locus and chose the one fulfilling the mixture ratio expectation. If there are two or more genotype combinations fulfilling the mixture ratio expectation, the DNA profile at that locus will either include a "Z" or be deemed inconclusive.

d. For some samples, the DNA profile of the minor contributor may also be deconvoluted. The DNA profile of the major contributor and the mixture ratio expectation should be used, as well as the specific guidelines described in the following sections. In order to facilitate this process, it may be useful to amplify the sample with more DNA, if sufficient DNA is available.

e. The DNA profile of an assumed contributor may be used to determine the most likely profile of another contributor. In this situation, the PHRs of the assigned contributors should meet the specific guidelines described in the following sections, taking potential allele sharing into account. Examples of assumed contributors include the following:

i. Examples of assumed contributors include the following:

1) A victim that is expected to have contributed biological material to the sample, and those DNA alleles are seen in the mixed sample.

2) An elimination sample such as a boyfriend, family member, or witness, and those DNA alleles are seen in the mixed sample.

3) A previously determined profile present in another sample within the case, and those DNA alleles are seen in the mixed sample.

ii. The report must state this assumption as follows: "*Assuming that (insert name A here) is a contributor to this mixture,…*" refer to the "STR Comparisons" procedure for further details.

Back to Table of contents

4. The first step in mixture deconvolution is to determine whether the sample meets the concordance policy.

a. A single amplification that fulfills the concordance policy and is suitable for deconvolution may be used. However, in order to deconvolute samples amplified with less than 250 pg of DNA template, duplication should be attempted with the following exceptions.

i. If a known donor is assumed to be one of the contributors to a concordant mixture and this known profile is utilized in the deconvolution (refer to section VII D for details), duplication is not required.

ii. Moreover, concordant mixtures used for comparison only do not need to be duplicated.

b. In order to fully resolve components of mixtures at loci which are saturated according to the Genemapper software, samples should be re-injected at a dilution or a lower parameter.

c. If multiple injections of a given PCR product and/or amplifications with varying amounts of DNA are generated for a sample, for each locus select the injection or amplification that shows the greatest number of labeled peaks that are not off scale or oversaturated.

i. For example, if a small locus is off scale in the first injection but is within range in the second injection, data from the second injection may be used for that locus.

ii. Similarly, if a large locus generates more data from the first injection than another, the data from the first injection may be used for that locus.

d. If duplicate amplifications are performed with the same DNA template amount follow the specific guidelines below for deconvolution.

5. The second step in analysis is to estimate the number of contributors to the sample.

a. A minimum number of contributors to a mixed profile can be estimated using the locus or loci demonstrating the largest number of labeled peaks.

b. At least two contributors:

i. If there are three or more labeled peaks at a locus, the sample may be considered to have at least two contributors.

1) Consider whether one of the peaks could be attributed to stutter.

Back to Table of contents

2) A third labeled peak at only one locus may be an indication of a tri-allelic pattern.

3) If an additional allele is present at only one or two loci, these alleles may be the result of a low level mixture detected only at those loci. The source of these allele(s) cannot be determined. The sample may be interpreted according to the guidelines for single source samples.

ii. Other indications of a two person mixture include observed peak height ratios between a single pair of labeled peaks at several loci below 60.5%. Tables 10a and 10b illustrate the empirically determined heterozygous PHR for single source samples.

c. At least three contributors:

Five alleles (repeating or non-repeating) are present at at least two loci. Stutter and other explainable artifacts should be considered when counting the number of alleles at a locus

If the analyst cannot decide between two and three contributors after applying the above guidelines, the table below can be considered. However, the analyst's discretion should be used when doing this determination. The entire sample should be taken into account when determining the number of contributors, which may include possible stochastic effects (e.g. peak height imbalance, drop in, etc).

HT-DNA Mixtures
≥ 2 loci with ≥ 5 different alleles
≥ 8 loci with ≥ 4 different alleles

Table 9. Characteristics of HT DNA mixtures with at least three contributors from Forensic Biology study (Perez et al CMJ 2011:393-405).
* Note that these characteristics were not seen for all three person mixtures in the study.

Back to Table of contents